ELECTRICAL STIMULATION RESEMBLING NORMAL MOTOR-UNIT ACTIVITY: EFFECTS ON DENERVATED FAST AND SLOW RAT MUSCLES

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SUMMARY

1. The slow-twitch soleus muscle and the fast-twitch extensor digitorum longus muscle (EDL) were denervated and stimulated directly with implanted electrodes for 33–82 days. Four different stimulation patterns were used in order to mimic important characteristics of the natural motor-unit activity in these muscles. In addition, to compare the effects of direct stimulation to other experimental models, some EDLs were stimulated through the nerve or cross-innervated by soleus axons.

2. After 33–82 days of stimulation the contractile properties were measured under isometric and isotonic conditions.

3. 'Native' stimulation patterns could maintain normal contractile speed in both EDL and soleus. In the EDL, normal isotonic shortening velocity was maintained only by a stimulation pattern consisting of very brief trains with an initial short interspike interval (doublet), and not by the other 'native' high-frequency patterns.

4. The contractile properties of both EDL and soleus muscles receiving a 'foreign' stimulation pattern were transformed in the direction of the muscle normally receiving that type of activity. The transformations were not complete, and soleus and EDL muscles stimulated with the same stimulation pattern remained different. This suggests that adult muscle fibres in rat EDL and soleus are irreversibly differentiated into different fibre types earlier in development.

5. The three high-frequency stimulation patterns used differed in their ability to change or maintain various contractile properties in the soleus and the EDL. The results indicate that the following qualities of a stimulation pattern might be of importance for the control of contractile properties: instantaneous frequency, total amount of stimulation, train length, interval between trains and presence of an initial doublet.

6. With the exception of the EDL shortening velocity, changes in contractile speed induced by a 'foreign' stimulation pattern were quantitatively similar to the effects of cross-innervation both in the EDL and the soleus. We thus suggest that the change in activity pattern is the mechanism behind most of the changes induced by cross-innervation.

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INTRODUCTION

Skeletal muscle fibres can be divided into a number of categories or 'fibre types', each of which is characterized by a set of contractile and molecular properties. On the basis of physiological properties single motor units have been classified into three main types, namely S (slow), FR (fast fatigue resistant) and FF (fast fatigable) (Burke, 1981; see Close, 1967, and Edström & Kugelberg, 1968, for measurements on rat motor units). Within these types there is further variability in physiological and molecular properties (Burke, 1981; Staron & Pette, 1987a, b). The diversity of muscle cells seems to be related to the motoneurones, because when the nerves to fast and slow muscles are cross-transplanted the muscle properties change according to the new nervous supply (Buller, Eccles & Eccles, 1960; Close, 1969). Lømo, Westgaard & Dahl (1974) demonstrated that this nervous influence could be due to the differences in the activity pattern in nerves to fast and slow muscles. They implanted electrodes on the denervated slow soleus muscle and stimulated it with a high-frequency pattern. This procedure decreased the twitch duration considerably. Several other authors have demonstrated the importance of activity by imposing artificial stimulation through the nerve (for review see Pette, 1984). This procedure, however, does not exclude the possibility that the changes in contractile properties are secondary to neuronal changes.

Recently the activity patterns of single motor units in the slow soleus muscle and the fast extensor digitorum longus muscle (EDL) of freely moving rats have been characterized in detail by Hennig & Lømo (1985). These observations provide important new information for the experimental simulation of naturally occurring activity in the nerves innervating different fibre types. The activity patterns could be classified into three distinct types presumably corresponding to S, FR and FF motor units. The presumed S units generated a large number of impulses grouped in long and short trains with median frequencies around 20 Hz, about twice the 10 Hz used as a 'slow' stimulation pattern in previous studies in the rat (Lømo et al. 1974; Lømo, Westgaard & Engebretsen, 1980). The presumed FF and FR units both fired at high frequencies, but differed dramatically in the total number of impulses delivered over 24 h (upper part of Table 1). In particular, the presumed FF units generated trains that were always brief and that usually started with a short interspike interval ('initial doublet'). Initial doublets augment the rate and magnitude of force development (Burke, Rudomin & Zajac, 1976; Hennig & Lømo, 1987b), but their role in the long-term regulation of contractile properties has not been investigated.

In the present paper we have removed all neural influence by denervation, and then stimulated the muscle fibres chronically with four different stimulation patterns in order to mimic some of the typical features of the normal soleus and EDL motor-unit activity. We have stimulated both the soleus muscle which consists mainly of slow fibres, and the EDL muscle which consists mainly of fast fibres. This allows a comparison of the effects of the same stimulation pattern on muscle fibres which start out with completely different properties.

We here present data on physiological properties while histochemical and biochemical data are presented elsewhere (Gundersen, Leberer, Lømo, Pette &

Staron, 1988). Preliminary reports of some of the data have been presented at meetings (Gundersen, Eken, Kardel & Lømo, 1984; Eken & Gundersen, 1984*b*; Gundersen & Eken, 1985).

METHODS

Material

The muscles studied were the fast-twitch extensor digitorum longus (EDL) and the slow-twitch soleus from hindlimbs of male Møll–Wistar rats. The rats weighed 240–530 g when the terminal experiment was performed. All operations and terminal experiments were done under Equithesin (a mixture of chloralhydrate and pentobarbitone) anaesthesia, 0·4 ml per 100 g body weight I.P.

Stimulation procedure

The operation and stimulation procedures were essentially the same as described by Gorza, Gundersen, Lømo, Schiaffino & Westgaard (1988). The leg was denervated by cutting and reflecting the sciatic nerve in the thigh. In the same operation multistranded steel electrodes were implanted on the muscles. In some rats pairs of electrodes were implanted both on the EDL and the soleus, and in these cases both muscles were stimulated simultaneously. In other rats only one of the muscles was stimulated, but this difference in implantation and stimulation procedure did not significantly influence the contractile properties we measured. The wires were taken from the head and connected to rotating contacts above the rat. This allowed the animals to move freely in large buckets during the stimulation. The electric stimulation was started 1–3 days after the operation, in order to let the rats recover and the intramuscular nerves to degenerate. The stimulator produced bipolar pulses that lasted 0·2 ms in each phase. The current was adjusted to 5–10 mA, which evoked clearly palpable, strong contractions in the soleus and the EDL, but without any pain responses from the rat. The stimulation was delivered continuously day and night.

In two rats the nerve was not cut, and the muscles were stimulated through the nerve. The stimulation procedure was similar to that of the direct stimulation, except that the electrodes were implanted in the vicinity of the intact sciatic nerve in the thigh, and that a smaller stimulation current of approximately 0·5 mA was used. In this situation the muscle is probably activated both by the electrical stimulation and by the endogenous activity from the central nervous system (Hennig & Lømo, 1987*a*).

We have included data from fifty-three muscles that were successfully stimulated for 33–82 days (mean 63 days). A preceding longitudinal study in which the denervated rat soleus was stimulated at 100 Hz showed that most of the transformation processes in the soleus occurred during the first month and that little further development occurred when the stimulation period was prolonged to 4 months (Gorza *et al.* 1988). In agreement with this we have observed no dependence on the length of the stimulation time in this study.

Stimulation patterns

Some characteristics of the three EMG patterns described by Hennig & Lømo (1985) are given in the upper part of Table 1. We chose to mimic these patterns with three different stereotyped stimulation paradigms: (1) 20 Hz trains of 200 pulses every 30 s (referred to as '20 Hz'); (2) 150 Hz trains of 25 pulses every 15 s ('150 Hz high-amount'); (3) 150 Hz trains of 25 pulses every 15 min ('150 Hz low-amount').

The characteristics of these stimulation patterns are summarized in the lower part of Table 1. As can be seen, the stimulation patterns were to some extent chosen to be caricatures rather than naturalistic imitations of motor-unit activity.

To mimic the brief trains and the presence of the initial doublet in the presumed FF units (Hennig & Lømo, 1985) a fourth stimulation pattern was designed: (4) three pulses with a first interspike interval of 5 ms followed by an interval of 10 ms. This train was delivered every 100 s (referred to as the 'triplet' pattern).

Cross-innervation

The soleus and fibular nerves were cut, and the soleus nerve was then sutured to the peripheral fibular nerve stump in order to let the soleus axons reinnervate the EDL muscle. Three successful cross-innervation experiments were performed, and 87–105 days after the operation the muscles were examined as described below.

TABLE 1. Comparison of normal activity patterns in motor units (Hennig & Lømo, 1985) and the electrical stimulation patterns used in this study (see Methods)

	Motor-unit activity patterns		
Presumed type	S	FR	FF
Frequency quartiles (Hz)	12–29	40–100	50–111
Impulses/ 24 h	309 500–495 800	89 500–243 100	2 600–11 200

	Mimicry stimulation patterns			
Pattern name	20 Hz	150 Hz high-amount	150 Hz low-amount	Triplet
Frequency (Hz)	20	150	150	200, 100
Pulses/ 24 h	576 000	144 000	2 400	2 592

Terminal experiment

After the chronic treatment the contractile properties of the muscles were examined in a terminal experiment. The muscle was exposed to allow the distal tendon to be connected to the recording device. Precautions were taken to keep the main blood supply intact. The skinned leg was submerged in 35 °C oxygenated Ringer solution and the muscle was stimulated supramaximally by an electric field from two platinum electrodes. The same stimulation method was used for innervated muscles, but in this case the muscle fibres were probably largely excited indirectly by their intramuscular nerves. All contractile properties were measured at the optimal length as determined by twitch force.

At the end of each experiment the muscles were exposed more completely while still connected to the recording device at their optimal length. They were then observed through the dissection microscope and the fibre lengths were measured as the distance from the most proximal point on the distal tendon to the point where the most proximal fibres insert into the proximal tendon. In individual muscles all fibres were assumed to be of the same length (Close, 1964).

The recording device was a pneumatic isotonic lever with an equivalent mass of 140 mg (Gundersen, 1985). The analog signals from the force transducer were fed into an Apple II+ microcomputer via an analog–digital converter and analysed on-line by a specially designed computer program (Eken & Gundersen, 1984a).

Analysis of force–velocity data

Force–velocity curves were calculated by fitting the measurements to the Hill (1938) equation:

$$(P+a) V = b(P_0 - P),$$

where P = load (force), V = shortening velocity and P_0 = maximal isometric tetanic force.

The curves shown in Fig. 4 were fitted according to Katz (1939). The velocity at zero load (V_{max}), given in Fig. 5 and Tables 2 and 3, was calculated according to Edman, Mulieri & Scubon-Mulieri (1976). This calculation represents a fitting of all the three constants (a, b and P_0) in the Hill (1938) equation. The calculated V_{max} was thus independent of the measured P_0, and this reduced the variability. In six out of fifty-four muscles the P_0 calculated from the force–velocity data was less than 80% of the measured P_0, and the calculations then tended to give an uncharacteristically low V_{max}. These muscles were excluded.

Statistics

The two-tailed Student's t test was used for statistical analysis. When $P > 0.05$ the result was considered not to be statistically significant.

Force and atrophy

Long-term denervation leads to weak, atrophic muscles, but this can to a large extent be counteracted by electrical stimulation (e.g. Hennig & Lømo, 1987a). The present study confirms this, and the maximal tetanic forces exerted by our stimulated muscles are illustrated in Fig. 1. There were no significant differences in
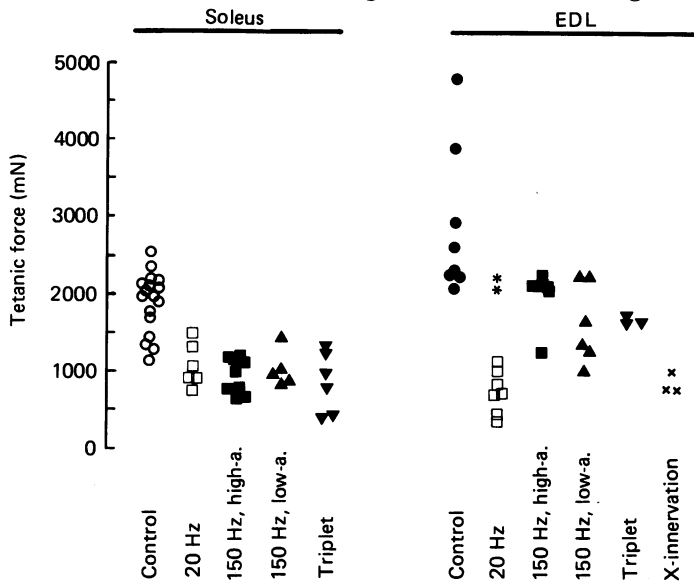

Fig. 1. Single observations of maximal tetanic force. ○, control solei; ●, control EDLs. Muscles stimulated by 20 Hz directly (□), 20 Hz via the nerve (*), 150 Hz high-amount (■), 150 Hz low-amount (▲), and triplet pattern (▼). ✕, EDLs cross-innervated by the soleus nerve.

the ability of the different stimulation patterns to maintain muscle strength, with one exception: EDLs stimulated with the 'foreign' 20 Hz pattern were significantly weaker than muscles stimulated by any of the other stimulation patterns ($P \leqslant 0.002$). On average the 20-Hz-stimulated EDLs also had the lowest muscle mass, cross-sectional area and intrinsic force (force per cross-sectional area) (Table 3). In the soleus the 20 Hz pattern, which mimics the 'native' activity of this muscle, was on average the most effective stimulation pattern in preventing atrophy and force loss (Table 2). However, the superiority to the 'foreign' high-frequency patterns was not statistically significant.

Twitch duration

Twitch time-to-peak and half-relaxation time are the most commonly used criteria for distinguishing between fast and slow muscles. As illustrated in Fig. 2 the twitch time-to-peak and half-relaxation time were essentially preserved by the 'native' stimulation patterns (20 Hz in the soleus; 150 Hz or triplets in the EDL) and changed by the 'foreign' stimulation patterns (150 Hz or triplets in the soleus; 20 Hz in the EDL). Thus, in the soleus the 'native' 20 Hz stimulation led to twitch time-to-peak values not significantly different from the control group although the
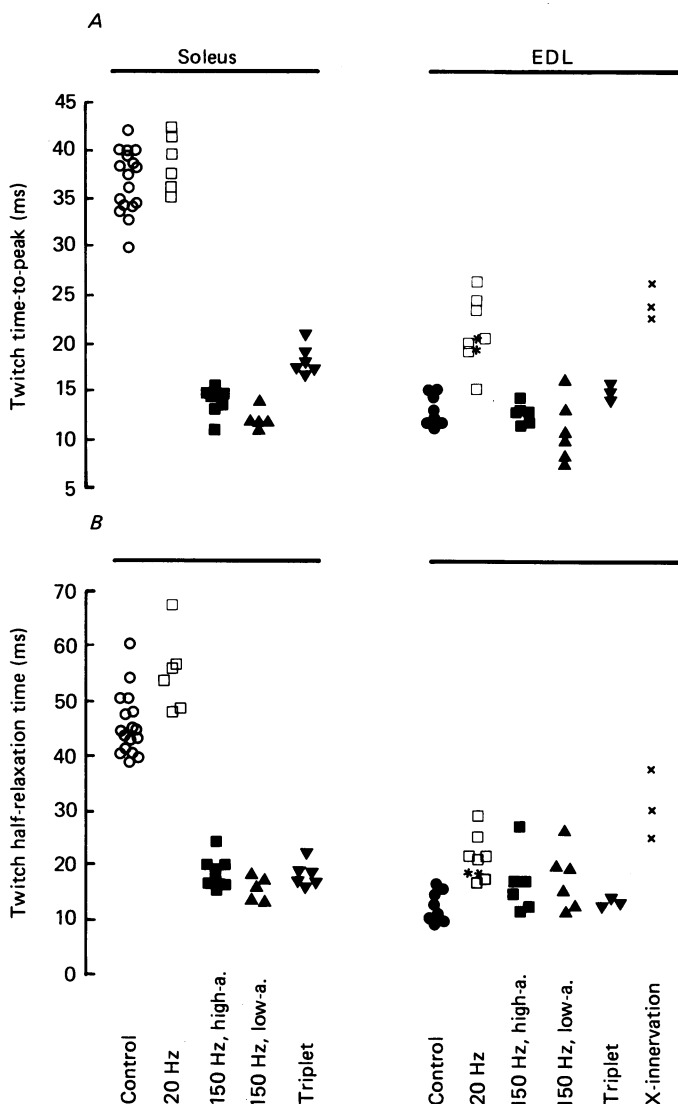
Fig. 2. Single observations of twitch time-to-peak (*A*), and twitch half-relaxation time (*B*). Symbols as in Fig. 1.

average half-relaxation time was somewhat prolonged (Fig. 2*B*). Similarly, the twitch duration in EDLs stimulated by any of the 'fast' stimulation patterns was not significantly different from the control group with the exception of the longer average half-relaxation time of muscles stimulated by the 150 Hz low-amount pattern (Fig. 2*B*; Table 3).

By contrast, stimulating soleus muscles with either of the 'foreign' 150 Hz patterns induced a twitch time-to-peak as short as in normal EDLs or EDLs stimulated with the same 150 Hz patterns (Fig. 2*A*). The 150 Hz low-amount stimulation led to solei that had slightly shorter time-to-peak values than with
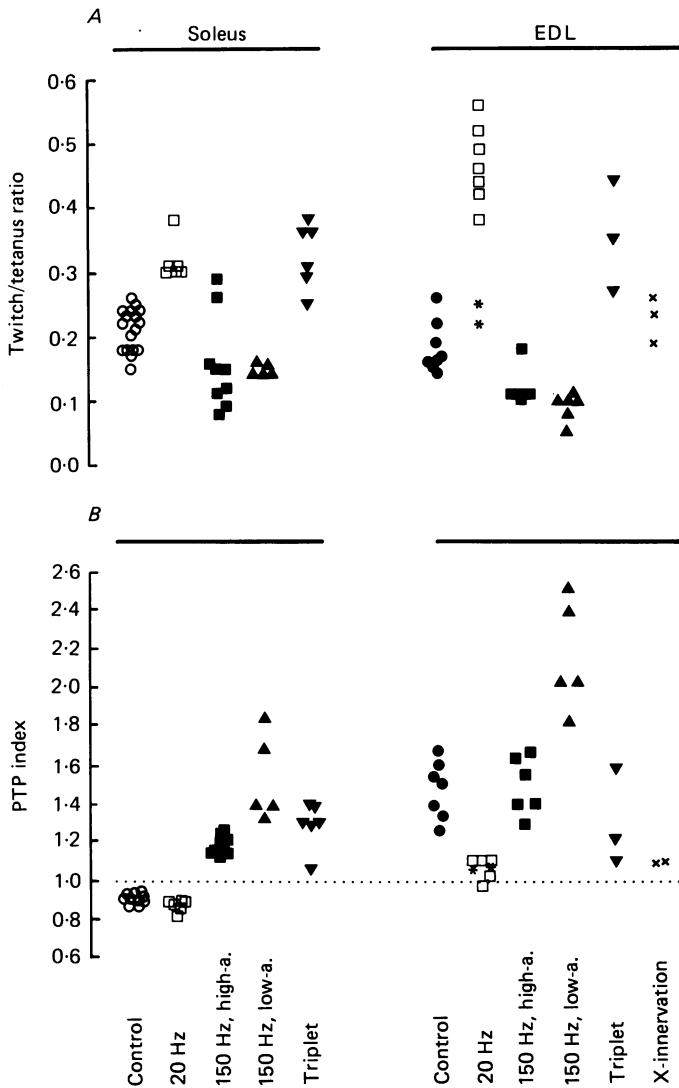
Fig. 3. *A*, single observations of twitch/tetanus force ratios. *B*, single observations of PTP indices (see Results for definition). A demarcation line between post-tetanic potentiation and depression is drawn. One PTP index value of 3·5 for an EDL muscle stimulated with the 150 Hz low-amount pattern is not shown. Symbols as in Fig. 1.

150 Hz high-amount stimulation ($P = 0·020$). Most striking, however, the triplets induced clearly higher time-to-peak values than both these patterns ($P = 0·001$). These high values were, however, not accompanied by correspondingly long half-relaxation times (Fig. 2*B*; Table 2).

Our findings in the soleus extend and confirm the data of Lømo *et al.* (1980). They were able to reduce the twitch time-to-peak of the soleus to 13·3 ms by a 100 Hz low-amount stimulation pattern and to 14·4 ms (mean values) when the amount was increased. These, and our findings, are in contrast to the data of Al-Amood, Finol &

Lewis (1986) who obtained a smaller degree of transformation (19·5 ms) after stimulation with a 100 Hz stimulation pattern.

In EDL, the 'foreign' 20 Hz stimulation prolonged the twitch time-to-peak to an average of 21·2 ms. This is significantly slower than in the control EDLs or EDLs stimulated by any of the fast stimulation patterns ($P \leqslant 0·02$) (Table 3). It is, however, not nearly as slow as the 36·7 ms found in the normal soleus or the 38·7 ms found in solei stimulated with the same 20 Hz pattern. The 21·2 ms in the 20-Hz-stimulated EDLs is on the other hand quite similar to the 24 ms found in a presumed type I motor unit in the normal EDL (Close, 1967). Therefore, with respect to the twitch time-to-peak, the fast-to-slow conversion of the EDL may be considered complete when compared to a slow motor unit from the same muscle, but not when compared to slow units in the soleus. A similar observation has been made in the cat where cross-innervation of the fast flexor digitorum longus muscle (FDL) by soleus axons resulted in motor units which were as slow as the normal FDL slow units, but not nearly as slow as the slow units in normal cat solei (Dum, O'Donovan, Toop & Burke, 1985; Dum, O'Donovan, Toop, Tsairis, Pinter & Burke, 1985).

Twitch/tetanus ratio

The twitch/tetanus ratio is thought to reflect the duration of the 'active state' of the contractile material during the twitch, so that a prolonged 'active state' leads to higher twitch/tetanus ratios and a shortened 'active state' to lower ratios (Close, 1972). All stimulation patterns led to significant changes in the twitch/tetanus ratio (Fig. 3*A*; Tables 2 and 3). In both EDL and soleus the 150 Hz patterns led to a decrease and the 20 Hz and triplet patterns to an increase in the twitch/tetanus ratio. This suggests that the 150 Hz stimulation shortened and the two other stimulation patterns prolonged the 'active state'. Such changes in the 'active state' may have contributed to the changes in the twitch duration, and particularly it might explain the relatively high twitch time-to-peak values obtained by the triplet pattern.

Post-tetanic potentiation (PTP)

When a tetanic stimulation is interposed in a series of twitches, it usually has a transient effect on the twitch force. The soleus normally shows a small post-tetanic force depression, whereas fast muscles become potentiated (Close, 1972). We have interposed a tetanus lasting 1 s in a series of twitches delivered every 3 s. The post-tetanic potentiation (PTP) index was calculated as the most potentiated (or in case of depression, the most depressed) post-tetanic twitch force divided by the pre-tetanic twitch force.

'Native' stimulation patterns could preserve normal values in both the soleus and the EDL, while 'foreign' stimulation patterns induced potentiation in the soleus and reduced potentiation in the EDL (Fig. 3*B*). The 150 Hz low-amount pattern induced extremely high PTP indices in both muscles. All the EDLs receiving this stimulation pattern had indices that were higher than in all the other EDL muscles (Fig. 3*B*). Solei receiving this stimulation pattern had PTP indices as high as in normal EDLs, but not as high as in EDLs stimulated with the same pattern.
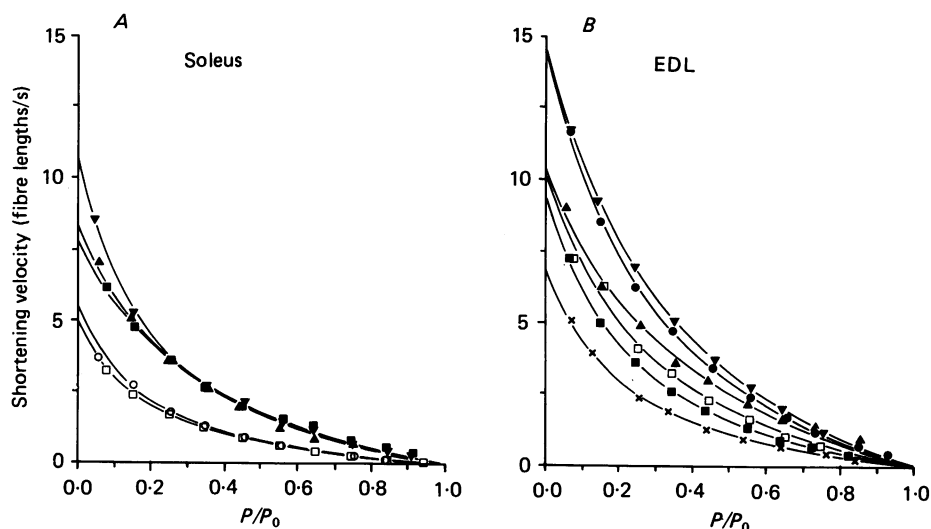
Fig. 4. Force–velocity curves from soleus (A) and EDL (B). Values from two to eight muscles were grouped into bins of $0.1\,P_0$ and mean values were calculated. The force–velocity curves were calculated by fitting the grouped data to the Hill (1938) equation. $\bigcirc$, control solei; $\bullet$, control EDLs. Muscles stimulated by 20 Hz directly ($\square$), 150 Hz high-amount ($\blacksquare$), 150 Hz low-amount, ($\blacktriangle$), and triplet pattern ($\blacktriangledown$). $\times$, EDLs cross-innervated by the soleus nerve.
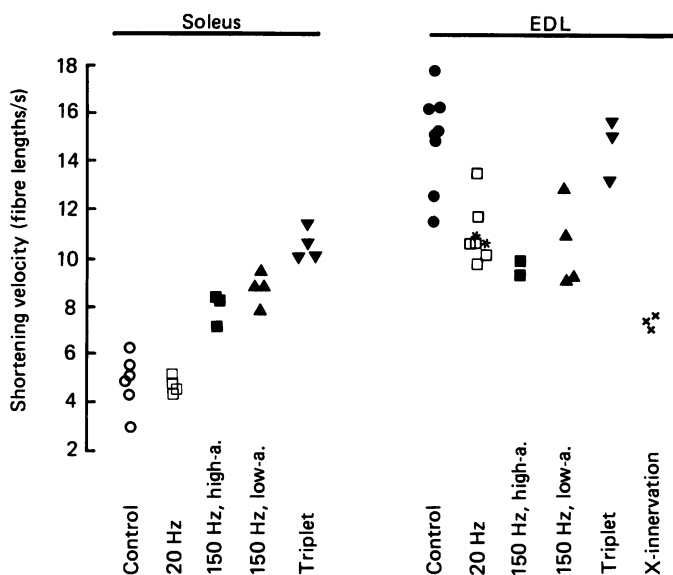

Fig. 5. Single observations of maximal intrinsic shortening velocities (V_{max}). See Methods. Symbols as in Fig. 1.

Isotonic shortening velocity

The isotonic shortening velocity reflects the sliding velocity between myosin and actin, and can be compared in different muscles provided the sarcomere length is the same, and that corrections are made for differences in fibre length (Close, 1972). Shortening velocity divided by fibre length (intrinsic shortening velocity) is illustrated in Figs 4 and 5. The main finding is that it was possible to maintain normal shortening velocities with the 20 Hz pattern in the soleus and the triplet pattern in the EDL. All the other stimulation patterns, including both 'native' 150 Hz patterns in the EDL, changed the intrinsic shortening velocity (Fig. 5).

The 'fast' stimulation patterns induced faster intrinsic shortening velocities in the soleus, but the muscles did not become as fast as the normal EDLs ($P = 0.031$). This finding is in agreement with the results of Al-Amood *el al.* (1986) and Gorza *et al.* (1988). Similarly the 'slow' 20 Hz pattern significantly reduced the intrinsic shortening velocity in the EDL (Fig. 5; Table 3), but the muscles did not become nearly as slow as normal solei. Moreover, the 20 Hz pattern was no more effective than the two 150 Hz patterns in reducing shortening velocity.

It is notable that the three different 'fast' stimulation patterns had different effects on the shortening velocity in the soleus. The triplet pattern was clearly the most effective ($P = 0.011$) and the 150 Hz low-amount pattern possibly was a little more effective than the 150 Hz high-amount pattern (not statistically significant). The triplet pattern was thus the most effective both in inducing and maintaining fast shortening velocity in soleus and EDL. This is remarkable since the triplets were the least effective of the 'fast' stimulation patterns in maintaining or inducing short twitch time-to-peak values (Fig. 2*A*).

While the intrinsic shortening velocity reflects the intrinsic properties of the contractile apparatus, the absolute shortening velocity of the whole muscle is more important from a functional point of view. With respect to this parameter the slow-to-fast conversion of the soleus by 'foreign' stimulation patterns was complete; it contracted at least as fast as the normal EDL (Tables 2 and 3). This was partly due to the increase in fibre length in the stimulated muscles (Table 2). A similar observation was made by Close (1969) after cross-innervating the soleus with the EDL nerve. He suggested that information might be conveyed from the muscle by the afferent nerves, and that the nervous system trophically adjusted the functional shortening velocity of the muscle rather than the intrinsic contractile properties. This explanation is ruled out in the present experiment because the muscles were denervated. The transformation of whole-muscle shortening velocity might simply be due to independent but concurrent changes in intrinsic shortening velocity and fibre length resulting in an apparent 'adjustment' of shortening velocity.

Endurance

Muscle endurance was tested at the end of each terminal experiment by stimulating the muscles repetitively at 77 Hz for 1/3 s every second. The fatigue index (Tables 2 and 3) was calculated as the ratio of the tetanic force after 2 min stimulation to the force at the beginning of the test (Burke, Levine, Tsairis & Zajac, 1973). There was great variability in these data, probably in part due to differences

in the blood supply to the partly exposed muscles. Nevertheless, muscle endurance seemed to be influenced by the total amount of activity; a high amount of activity led to less fatigable muscles while low amounts led to more fatigable muscles. Thus, in the soleus the 150 Hz low-amount pattern reduced endurance (Table 2), while in the EDL the 20 Hz and 150 Hz high-amount patterns increased endurance (Table 3). For the soleus this confirms the findings of Lømo *et al.* (1980), and we demonstrate in addition that the EDL responds similarly.

Apart from number of impulses, other aspects of the impulse patterns appear to be important for the control of endurance. Thus, in spite of consisting of about the same number of impulses (Table 1), the triplet pattern produced fatigue indices in the soleus that were on average twice as high as in solei stimulated by the 150 Hz low-amount pattern ($P = 0.027$).

Nerve stimulation

The limited transformation induced by the 20 Hz stimulation in the denervated rat EDL was remarkable considering that the rabbit EDL apparently is completely transformed by 'slow' stimulation delivered through the nerve (Salmons & Sréter, 1976). To investigate this difference further we stimulated two EDLs with the 20 Hz pattern through the nerve. It appeared that the presence of the nerve had little effect on the fast-to-slow conversion of the rat EDL. Twitch duration, shortening velocity, fatigability, and PTP indices were all very similar to those of denervated muscles stimulated directly with the same pattern (compare open squares and asterisks in Figs 2, 3*B* and 5; Tables 2 and 3). On the other hand the degree of atrophy and the twitch/tetanus ratio were significantly different in EDLs stimulated directly and via the nerve (Figs 1 and 3*A*; Table 3). The more pronounced atrophy in the denervated muscles could be due to local tissue damage or incomplete activation of all the muscle fibres during direct stimulation (Hennig & Lømo, 1987*a*; Gorza *et al.* 1988; Gundersen *et al.* 1988). The presence of the nerve *per se* might also play a role either through putative neurotrophic substances (Spector, 1985), or by transmitting normal high-frequency activity from the central nervous system. High-frequency background activity in the nerve could also explain the lower twitch/tetanus ratios in nerve-stimulated EDLs (asterisks in Fig. 3*A*), since such activity tends to reduce the twitch/tetanus ratio (present data, and Eerbeek, Kernell & Verhey, 1984). Alternatively the low values could be attributable to an increase in the number of subthreshold synapses after chronic 'foreign' stimulation, such that more fibres were activated in a tetanus than in a twitch during the terminal experiment (Dudel & Kuffler, 1961; Bárány & Close, 1971; Fladby, 1987).

Cross-innervation

Comparison of the effects of direct stimulation with the effects of cross-innervation obtained by others (Close, 1969; Bárány & Close, 1971), revealed that the effects on contraction speed were similar except for the fast-to-slow transformation of the EDL shortening velocity (see Discussion). To determine whether or not this exception was reproducible we repeated the cross-innervation experiment for the EDL. Our measurements from EDL muscles innervated by the soleus nerve (crosses in Figs 1–5; Table 3) essentially confirmed the earlier data (Close, 1969; Bárány & Close,

TABLE 2. Properties of normal and stimulated soleus muscles

	Control	20 Hz	150 Hz high-amount	150 Hz low-amount	Triplet
Muscle mass (mg)	186±45 (18)	157±48 (6)	143±19***†† (8)	126±12** (4)	131±32** (6)
Relative muscle mass (mg/kg)[a]	485±91 (18)	421±113† (5)	350±32***†† (6)	333±26** (4)	348±77** (5)
Fibre length (mm)	18·0±2·0†† (18)	20·7±1·7*†† (4)	24·5±3·0***†† (9)	24·2±2·7***†† (5)	22·3±2·8***†† (6)
Cross-sectional area (mm²)[b]	10·4±2·5†† (17)	7·5±2·2* (4)	5·8±0·8***†† (8)	5·4±0·7***†† (4)	5·9±1·1***†† (6)
Tetanic force (mN)	1879±392†† (17)	1077±278** (6)	955±225***†† (9)	1036±245*† (5)	872±383·2***†† (6)
Intrinsic tetanic force (mN/mm²)[c]	171±37 (12)	137±12 (4)	162±37 (8)	199±40 (4)	149±61 (6)
Twitch force (mN)	395±86 (17)	336±79 (6)	147±50***†† (9)	154±33** (5)	280±116***†† (6)
Twitch/tetanus force ratio	0·21±0·03 (17)	0·32±0·03***†† (6)	0·16±0·07** (9)	0·15±0·01***†† (5)	0·32±0·05** (6)
Twitch time-to-peak (ms)	36·7±3·3†† (17)	38·7±3·0†† (6)	14·0±1·3** (9)	12·2±1·1** (5)	18·3±1·5***†† (6)
Twitch half-relaxation time (ms)	45·3±5·7†† (17)	54·7±7·0***†† (6)	18·2±2·7** (9)	15·7±2·3** (5)	18·4±2·3***†† (6)
V_{max} (mm/s)[d]	81·3±15·5†† (7)	100·4±8·2*†† (5)	182·9±13·3** (3)	206·9±31·5** (4)	237·7±32·8** (4)
Intrinsic V_{max} (fibre lengths/s)[e]	4·8±1·1†† (6)	4·7±0·4†† (4)	8·0±0·6***† (3)	8·8±0·7** (4)	10·5±0·7***†† (4)
a/P_0^f	0·24±0·05 (7)	0·23±0·06† (5)	0·33±0·09 (3)	0·30±0·05 (4)	0·30±0·12 (4)
Post-tetanic potentiation index[e]	0·90±0·02†† (14)	0·87±0·03†† (6)	1·19±0·06***†† (9)	1·52±0·23***†† (5)	1·29±0·12** (6)
Fatigue index[e]	0·58±0·24†† (14)	0·71±0·14 (6)	0·41±0·26 (9)	0·15±0·06** (5)	0·37±0·16 (4)

Mean ±S.D. (number of observations in parentheses). * and **, significantly different from controls; † and ††, significantly different from similarly treated EDLs. Single symbols, $P \leqslant 0·05$; double symbols, $P \leqslant 0·02$. [a] Muscle mass divided by animal body weight. [b] Calculated according to Close (1972). [c] Force divided by cross-sectional area. [d] See Methods. [e] See Results for definition. [f] Parameter indicating the curvature of the force–velocity curve (Close 1972).

TABLE 3. Properties of normal and stimulated EDL muscles

	Control	20 Hz	150 Hz high-amount	150 Hz low-amount	Triplet	20 Hz via the nerve	Cross-innervation
Muscle mass (mg)	184±35 (13)	118±18** (6)	199±42†† (6)	156±39 (6)	155±11 (3)	176±11†† (2)	123±28* (2)
Relative muscle mass (mg/kg)[a]	496±52 (13)	290±36***† (5)	448±68†† (6)	395±77** (6)	378±34** (3)	347±42** (2)	272±69** (2)
Fibre length (mm)	13·4±1·2†† (12)	15·6±2·5†† (6)	17·3±1·3***†† (6)	16·7±2·7***†† (5)	15·9±1·0***†† (3)	14·7±0·92 (2)	14·8±2·0 (3)
Cross-sectional area (mm²)[b]	13·4±2·4†† (12)	7·9±1·6** (5)	11·6±2·5†† (6)	9·6±2·3***†† (5)	9·8±1·2†† (3)	12·0±0·0†† (2)	9·0±2·6* (2)
Tetanic force (mN)	2901±956†† (8)	770±288** (7)	1993±358***†† (6)	1665±509***† (6)	1712±50†† (3)	2153±100†† (2)	890±135** (3)
Intrinsic tetanic force (mN/mm²)[c]	186±57 (6)	100±40** (5)	174±25 (6)	173±34 (5)	176±24 (3)	180±8† (2)	94±26 (2)
Twitch force (mN)	505±197 (8)	349±118 (7)	227±11***†† (6)	154±64** (6)	567±83†† (3)	508±70 (2)	222±68* (3)
Twitch/tetanus force ratio	0·18±0·04 (8)	0·47±0·06***†† (7)	0·12±0·03** (6)	0·09±0·02***†† (6)	0·35±0·09** (3)	0·24±0·02†† (2)	0·23±0·04†† (3)
Twitch time-to-peak (ms)	13·1±1·6†† (8)	21·2±3·7***†† (7)	12·8±1·1 (6)	11·1±3·2 (6)	14·9±0·8†† (3)	19·8±0·6** (2)	24·3±1·6** (3)
Twitch half-relaxation time (ms)	12·5±2·0†† (8)	21·8±4·2***†† (7)	16·7±5·5 (6)	17·5±5·4* (6)	13·5±0·8†† (3)	18·1±0·4 (2)	31·0±6·23***† (3)
V_{max} (mm/s)[d]	201±19†† (8)	171±25***†† (6)	169±21 (2)	188±50 (4)	232±5* (3)	156±10** (2)	111±11***†† (3)
Intrinsic V_{max} (fibre lengths/s)[e]	14·9±2·0†† (8)	11·1±1·4***†† (6)	9·6±0·4**† (2)	10·6±1·7** (4)	14·7±1·25†† (3)	10·7±0·1 (2)	7·5±0·3***†† (3)
a/P_0[f]	0·31±0·10 (8)	0·36±0·10† (6)	0·24±0·02 (2)	0·38±0·08 (4)	0·40±0·08 (3)	0·25±0·02 (2)	0·19±0·05† (3)
Post-tetanic potentiation index[e]	1·47±0·15†† (7)	1·07±0·05***†† (7)	1·48±0·15†† (6)	2·39±0·62***†† (6)	1·30±0·25 (6)	1·07±0·01** (2)	1·09±0·01** (2)
Fatigue index[e]	0·23±0·14†† (5)	0·51±0·21* (7)	0·45±0·14* (5)	0·28±0·17 (6)	0·23±0·01 (3)	0·36±0·18 (2)	0·60±0·07** (3)

Mean±s.d. (number of observations in parentheses). * and **, significantly different from controls; † and ††, significantly different from similarly treated solei; ‡ and ‡‡, significantly different from denervated, 20-Hz-stimulated EDLs. Single symbols. $P \leq 0·05$; double symbols, $P \leq 0·02$.

[a] Muscle mass divided by animal body weight. [b] Calculated according to Close (1972). [c] Force divided by cross-sectional area. [d] See Methods. [e] See Results for definition. [f] Parameter indicating the curvature of the force–velocity curve (Close 1972).

1971). Thus, most of the effects of the 'foreign' nerve are similar to the effects of mere 'foreign' stimulation, but the intrinsic shortening velocity is an exception (compare crosses and open squares in Figs 4 and 5; see Table 3 for statistics). Another exception is the twitch/tetanus ratio which was increased after denervation and 20 Hz stimulation, but apparently left unchanged by cross-innervation (Fig. 3A; Table 3). According to Close (1969, 1972) an unchanged twitch/tetanus ratio is exactly what one would expect if both twitch speed and shortening velocity were reduced proportionately, as was the case in our cross-innervation experiments. One should, however, also consider whether the relatively low ratios in cross-innervated muscles could be explained by failure of synaptic transmission in some regenerated synapses during a single twitch (Dudel & Kuffler, 1961; Bárány & Close, 1971; Fladby, 1987).

DISCUSSION

'Native' stimulation patterns preserved normal contractile properties in denervated muscles

Our data show that normal twitch duration and shortening velocity can be maintained in the denervated EDL and soleus by electrical stimulation with patterns resembling the normal motor-unit activity in these muscles. Others have reported that high-frequency stimulation fails to maintain normal properties of fast muscles (Sréter, Pinter, Jolesz & Mabuchi, 1982; Hudlická, Tyler, Srihari, Heilig & Pette, 1982; Eerbeek et al. 1984), and we failed to maintain normal shortening velocity in the EDL with either 150 Hz pattern. The successful maintenance of normal properties with the triplet pattern suggests that the patterning of impulses is critical. This stimulation pattern mimics dominant features of normal fast-motor-unit activity, as up to half of the firing episodes in presumed FR and FF motor units consist of as little as one to three action potentials, and more than 70% of the episodes in presumed FF units start with an initial doublet (Hennig & Lømo, 1985).

'Foreign' stimulation patterns induced incomplete changes in contractile properties

'Foreign' stimulation patterns induced changes in contractile properties of both the soleus and the EDL. The soleus became faster, whereas the EDL became slower. These changes can be regarded as adaptive since they tended to preserve the functionally important match that normally exists beween imposed activity and contractile properties (Kernell, 1979; Hennig & Lømo, 1985). However, the transformations were incomplete because the 'slow'-stimulated EDLs did not acquire the same properties as the normal soleus, nor did the 'fast'-stimulated solei acquire the same properties as the normal EDL. Moreover, EDLs and solei receiving the same stimulation patterns remained different (see Tables 2 and 3).

The incomplete fast-to-slow conversion of physiological properties in the 'slow'-stimulated rat EDL is in contrast to the apparently complete changes in twitch duration and myosin light chains reported in the rabbit EDL after 'slow' nerve stimulation (Salmons & Sréter, 1976). An incomplete transformation could be due to a sub-optimal stimulation regime. It is unlikely in this regard that the rat muscles were not stimulated long enough. In the soleus little further transformation was

observed after the first month when the stimulation lasted for up to 4 months (Gorza *et al.* 1988), and in the present study there was apparently no dependence on the stimulation time in the EDLs stimulated for 50–71 days. Neither was the incomplete transformation observed in our directly stimulated rat EDLs due to the absence of the nerve, since it was similarly incomplete when we stimulated via the intact nerve. We conclude that there seems to be a true species difference between rabbit and rat with respect to the extent to which it is possible to change the EDL muscle by changes in activity. Since it is still debated whether the conversion in the rabbit is complete with respect to all properties (Staron, Gohlsch & Pette, 1987), this does not necessarily mean that a true fibre-type conversion is possible in the rabbit and impossible in the rat. The demonstration of a complete fibre-type conversion would be of great importance since it would suggest that muscle cells in adult animals are so plastic that the fibre-type composition can be determined exclusively by nerve activity. If, on the other hand, muscle cells cannot be completely converted, this suggests that they are irreversibly differentiated into fibre types earlier in development.

Recent data from the avian embryo support the idea of an early differentiation. In the chick the basic fibre-type composition in the limb seems to be laid down already at the time of innervation (Crow & Stockdale, 1986), and the early differentiation of muscle fibre types can occur even in the absence of the nerve (Phillips & Bennett, 1984). Furthermore Miller & Stockdale (1986) found that myoblasts in culture apparently comprise three lineages that differentiate into separate types of myotubes. Similar investigations in mammals would be of great interest. In the present work the soleus and the EDL responded differently to identical stimulation and could not be completely interconverted, suggesting that adult rat fast and slow muscle cells may indeed be irreversibly differentiated earlier in development.

The various 'fast' stimulation patterns had different effects

In both soleus and EDL the parallel changes in several properties like twitch time-to-peak, twitch half-relaxation time, isotonic shortening velocity and PTP index suggested that their regulation is coupled. This coupling, however, did not appear to be very tight. Some of the EDLs stimulated with the 150 Hz low-amount pattern had faster twitches but slower intrinsic shortening velocities than normal controls. Of the 'fast' stimulation patterns the triplets were the most effective in inducing a fast shortening velocity, but the least effective in shortening the twitch time-to-peak. The relatively long twitch time-to-peak in triplet-stimulated muscles was not coupled to correspondingly long half-relaxation times. The PTP indices were markedly higher after stimulation with 150 Hz low-amount than by any of the other stimulation patterns, but this pattern did not have similarly outstanding effects on other contractile properties. All these findings indicate that individual contractile properties can to some extent be controlled independently.

The three 'fast' stimulation patterns differ with respect to total amount of activity, train length, intervals between trains and the presence of an initial doublet. All these attributes may differ in their effect on various contractile properties. For example, 150 Hz high-amount stimulation resulted in greater resistance to fatigue than 150 Hz low-amount stimulation suggesting that the total amount of activity is

critical for the control of this parameter (see also Lømo *et al.* 1980). On the other hand, the two 150 Hz patterns prevented denervation atrophy to about the same extent in spite of the 60-fold difference in total number of impulses. For the control of contraction speed both frequency and total amount of activity have been proposed to be of importance; thus in the soleus high frequency leads to fast twitches while an
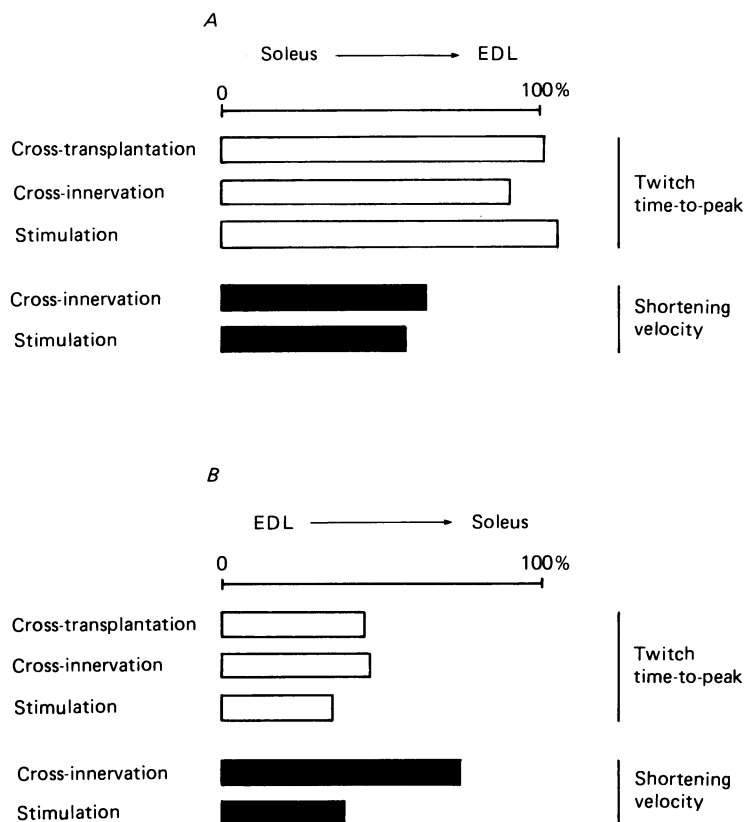

Fig. 6. The degree of transformation obtained by different experimental models, shown as the difference between the experimental and control values calculated as percentage of the difference between the soleus and EDL control values. Data on cross-transplantation are from Gutmann & Carlson (1975). Data on cross-innervation of the soleus are from Close (1969) and Bárány & Close (1971). The other calculations are based on the present data. The calculations on the effects of stimulation are based on the results from all muscles stimulated by that 'foreign' pattern which gave the highest degree of transformation.

increased amount reduces twitch speed (Lømo, Westgaard, Hennig & Gundersen, 1985). The present data indicate that also the particular patterning of impulses is important. Thus, the triplet pattern led to slower twitches and faster intrinsic shortening velocities than the 150 Hz low-amount pattern. The triplets have a similar mean frequency and only 8% more impulses, but differ in having shorter trains delivered more often, and in having initial doublets.

In conclusion, these data suggest that different properties can be controlled independently by several aspects of the activity patterns. This could partly explain

muscle fibre heterogeneity and suggests the possibility that the nervous system can fine tune muscle cell properties by imposed activity.

Effects of 'foreign' activity are similar to effects of 'foreign' innervation

Which signals cause the muscle fibre transformation? To answer this question it is of interest to compare the different experimental manipulations that induce changes. The classical model is the cross-innervation experiment (Buller *et al.* 1960; Close, 1969; Bárány & Close, 1971), but an even more radical attempt to change contractile properties has been made by Gutmann & Carlson (1975). They transplanted the EDL of rats into the position of the soleus while the soleus was transplanted into the place of the EDL. The transplanted muscles were sutured to the foreign tendons and reinnervated by the foreign nerves.

Both cross-innervation and cross-transplantation are complex experiments. The transplantation procedure involves muscle fibre degeneration and regeneration, and the transplanted muscle assumes completely the environment of the other muscle, including external load, putative neurotrophic substances and activity pattern. In cross-innervation experiments at least the two latter mechanisms might be involved (Buller *et al.* 1960). We have compared the degree of transformation obtained by these methods to that of direct electrical stimulation without the nerve being present (Fig. 6). The degree of transformation obtained by the three different experimental procedures was roughly the same for the slow-to-fast transformation of the soleus (Fig. 6*A*), and the fast-to-slow transformation of the EDL *twitch* (open bars in Fig. 6*B*). For the transformation of the EDL intrinsic shortening velocity there was, however, a clear difference between the experiments (Figs 4 and 5; filled bars in Fig. 6*B*). This suggests that the fast-to-slow transformation of the shortening velocity seen after cross-innervation in the rat EDL cannot solely be explained by changes in the activity pattern of the muscle fibres. Other mechanisms like neurotrophic substances or selective reinnervation with subsequent fibre loss might play a role. Alternatively the difference might be due to failure of mimicking the 'slow' activity pattern properly. The effects of different 'fast' stimulation patterns varied considerably, but we tried only one, highly stereotyped 'slow' stimulation pattern which might lack some critical features. For example, the presumed S motor units of Hennig & Lømo (1985) varied considerably in train length; more than half of the trains lasted less than a second, while the longest trains lasted for more than 5 min. The frequency was also not fixed at 20 Hz; more than 25% of the interspike intervals corresponded to frequencies less than 15 Hz. This illustrates the complexity and the difficulties in designing naturalistic imitations even of so-called 'tonic' motor-unit activity.

In conclusion, nearly normal properties can be maintained in denervated rat EDL and soleus muscles, provided activity resembling normal motor-unit activity is maintained. 'Foreign' stimulation patterns induce only limited transformation towards the fibre type corresponding to the activity pattern, but similar limitations apply to transformations obtained by cross-innervation. These findings indicate that evoked muscle cell activity can account for most of the control of contractile properties exerted by motoneurones.

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